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Milk Production of Cows on Protein-Free Feed

Studies of the use of urea and ammonium salts as the sole nitrogen source open new important perspectives.

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The cow has a key role as a producer of protein and also of many vitamins. Both milk and meat proteins are of high biological value. If the vegetable diet, containing mostly cereals, which is the normal diet of most of the world's population, could be supplemented by half a liter of milk per person per day, malnutrition would very nearly disappear. Since great losses occur when plant protein is changed into animal protein, it has been questioned whether there will be, in the future, any possibility of the production of animal protein in a more and more overcrowded world. In milk production the daily feed has to contain roughly 60 grams of digestible crude protein for each kilogram of milk produced, in addition to the 300 grams necessary for the maintenance of the cow. This is nearly twice as much protein as there is in 1 kilogram of milk. Because utilization of nutrients by the body in ruminants is very different from the utilization in other mammals, the fermentation processes in the rumen, caused by microbial flora, being of decisive importance, a part of the protein in the feed of ruminants can consist of simple nitrogen compounds—for example, amides or ammonia. This view was presented as early as 1891 by Zuntz (*1*) in Germany, but utilization of nonprotein nitrogen in the rumen is still not well understood. A great number of feeding experiments have been made in different countries to find out how much of the protein can be successfully replaced by urea, which is readily decomposed to ammonia in the rumen. The problem is complicated in the case of normal feeding with plenty of protein. This is due primarily to the fact that the microbes in the rumen more or less decompose the different proteins of the feed to ammonia and that microbial protein is again partly synthesized from the ammonia. There is danger of ammonia poisoning when urea is used as a supplement to normal feed, and thus the addition of only relatively small amounts of urea has been recommended in practice.

In experiments in which the purified feed used does not contain protein, and in which urea is used as the only significant source of nitrogen, the interpretation of the results is much clearer. Experiments of this kind have been carried out especially in the United States, with growing lambs, goats, and steers. The ruminal biosynthesis of all protein amino acids, even of essential amino acids, was demonstrated in these experiments (*2*). In spite of the relatively small amounts of protein which are needed for the growth of young ruminants—for example, for the rearing of a heifer, about 300 to 350 grams of digestible crude protein per day—optimum growth was not obtained with

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urea as the sole source of nitrogen. W. R. Woods and A. D. Tillman (*3*) noted the growth-promoting effect of an alkaline mineral mixture when purified diets with urea as the sole source of nitrogen were used. However, the growth of sheep was still inferior, when urea was used, to the growth obtained with soya protein (*3*). As may be seen from the foregoing findings, a cow producing, for instance, 15 kilograms of milk per day has to receive about 1200 grams of digestible crude protein. It is thus understandable that milk production on a protein-free feed has not been investigated. However, many problems —for example, the capacity of the microbial flora of the rumen to synthesize amino acids and proteins, the origin of milk flavor, and the biosynthesis of different milk components—actually require the study of milk production on a purified feed, with urea and ammonium salts as the sole sources of nitrogen. Such a study is of course fundamental also from a practical point of view.

Our studies on the production of milk with a purified, protein-free feed, with urea and small amounts of ammonium salts as practically the sole sources of nitrogen, were started in connection with a research project concerning the origin of the flavor substances of milk. The question under study was to what extent the flavor substances are due to synthesis within the cow's body and to what extent they are derived from the feed. It was thus important to prepare a test feed which was as simple and as pure as possible. Proteins were replaced by the simple nitrogen compounds urea and ammonium salts, and the complex carbohydrates and other organic compounds present in plants were replaced by purified starch, cellulose, and sucrose.

Feeding Experiments with Labeled Ammonia and Urea

In our feeding experiments performed in the spring of 1958, in which a cow on normal feed was given one dose of ammonium sulfate labeled with ^{15}N, the degree of labeling of the amino acids, separated by fractionation after hydrolysis of the milk proteins, was determined. It was shown that in the milk obtained 15 hours after the feeding of ^{15}N-ammonium sulfate all the protein amino acids studied were labeled, but that the degree of labeling of the various amino acids differed (*4*). The essential amino acids were labeled more weakly than the nonessential ones; this was natural, since the former are obviously formed only in the rumen by microorganisms, whereas the latter are also formed in other parts of the body, especially in the liver, from ammonia. When the labeling of glutamic acid, which was labeled more rapidly than any other amino acid, was taken as 100, the labeling of valine was 59; of lysine, 54; of phenylalanine, 50; of arginine, 41; of histidine, only 15.

This experiment demonstrated that the microbial flora of the rumen is able to synthesize all the protein amino acids and that the amino acids formed are used for the synthesis of milk protein. The weak labeling of some amino acids suggested that their synthesis may form a bottleneck in protein synthesis. Thus the possibility of developing in the rumen, by adaptation to a test feed, a microbial flora which could synthesize protein from ammonia more effectively than the rumen microorganisms of cows on normal feed and thus make milk production possible seemed to deserve experimental study.

The experiments on protein-free feed with urea nitrogen and ammonium nitrogen as the sole sources of nitrogen were started with one cow in the autumn of 1961 and with another at the beginning of 1962. A very slow adaptation procedure was used. When one of the test cows (Eiru) was given a dose of ^{15}N-labeled urea after having been on the test feed for 6 months and the labeling of the amino acids of milk protein was quantitatively determined 6.3 hours and 20 hours after the administration of ^{15}N, the labeling of essential amino acids generally, and especially of some of them, was found to be strongly increased relative to values obtained in experiments with a cow not adapted to the test feed. Another experiment, in which ^{15}N-ammonium sulfate was given the same test cow after it had been on test feed for 25 months, gave similar results. The results show that a dose of ^{15}N-urea and a dose of ^{15}N-ammonium sulfate labeled most of the amino acids of milk protein to practically the same degree, and, further, that the effects on labeling of feeding test feed for 6 months and for 25 months were similar. The curves in Fig. 1 illustrate the effect of adaptation to the test feed on the labeling of some essential amino acids of milk protein.

The labeling experiments with different cows on normal feed give results that differ quantitatively from one another, but the labeling of tryptophan (not estimated in the first experiment) and of histidine has always been the lowest of the values for labeling of the essential amino acids.

Test Feed and Milk Production

The composition of the test feed used in these experiments in two different time periods is given below (*5*).

1) Briquettes, about 9 grams each (paired figures in parentheses are percentages of dry substance for 1962–63 and 1965, respectively): α-cellulose powder (9.5; 9.9); starch (57.0; 52.9); sucrose (20.9; 23.1); salt mixture (8.2; 8.9); urea (94 percent) plus ammonium salts (6 percent), calculated as urea (4.4; 5.2). Water content, 15 percent. Besides these briquettes some test cows were occasionally fed sugar-free briquettes.

2) Wet cellulose paste mixed with water (paired figures in parentheses are percentages of dry substance for 1962–63 and 1965, respectively): α-cellulose powder (60.3; 57.3); starch (19.5; 16.4); sucrose (12.1; 12.2); salt mixture (7.1; 8.8); urea (1.0; 5.3). Water content, 75 percent.

3) Cellulose strips with precipitate of silicic acid, or without it. Water (75 percent); urea (4 percent of dry substance) and salt mixture (3 percent of dry substance).

4) Plant oils, 50 to 130 grams per cow per day.

5) Vitamins A and D: A (37,500 to 100,000 I.U. per cow per day) and D_2 plus D_3 (7500 to 20,000 I.U. per cow per day).

6) Vitamin E (α-tocopherol), 30 milligrams per cow per day in 1965; after that, 330 to 500 milligrams per cow per day. During the first years no vitamin E preparation was used.

7) Salt mixture. The composition of the salt mixture has been changed to some extent during the experiment on the basis of the analysis of feces. The mixture contains at present (in grams per measure of dry mixture): sodium, 6.9; potassium, 12.5; calcium, 14.4; magnesium, 5.3; chlorine, 11.0; sulfur (as sulfate), 3.8; phosphorus, 14.8; iron, 0.238; zinc, 0.085; manganese, 0.038; copper, 0.015; selenium, 0.003; boron, 0.003; cobalt, 0.001; iodine, 0.001; molybdenum, 0.0006.

Most of the time the starch used has been potato starch; this contains only 0.02 percent of nitrogen, whereas the maize starch contains 0.09 percent. The amount of nitrogen-containing impurities in the test feed is so small that the urea and ammonium nitrogen account for 99.5 percent of the total nitrogen.

Different test cows were given briquettes, cellulose-rich paste, and cellulose strips in different proportions according to appetite. Thus the feed of one cow differed from the feed of another to some extent. Roughly speaking it can be said that the test feed contained potato starch, cellulose, and sucrose in the following proportions: potato starch, 50 to 55 percent of the total carbohydrates; cellulose, 25 to 30 percent; sucrose, 17 to 23 percent. The success of the urea feeding is decisively dependent on the composition of the carbohydrates fed (*6*). The proportion of starch in the feed cannot be reduced very much without an accompanying drop in milk production.

In the adaptation of the cows to the test feed the amount of normal fodder was gradually decreased and the amount of test feed was correspondingly increased. The transfer to the test feed with the first two cows of our experiments was made over a period of 4 to 6 months; later on, with other cows, this period was reduced to 2 or 3 months. The test cows were fed twice a day. The cows producing large amounts of milk (more than 10 to 12 kilograms per day) ate their larger rations gradually all day long and regulated in this way the intake of urea. There was thus no need to divide the feed repeatedly into small portions. In contrast, the dry cows consumed their small rations in a very short time.

As indicated above, the nitrogen content of the feed has been raised considerably during the experiment. For fear of ammonia poisoning, urea and ammonium salts were at first used cautiously. The largest amount of urea (ammonium nitrogen included) used during the first 2 years was only a little more than 400 grams per day during the period of highest milk production. However, since analysis of the rumen contents and blood showed that ammonia had not accumulated in the rumen (*7*) and that the ammonia content of the blood had not risen, the amount of nitrogen in the feed was raised, beginning in 1964; since autumn 1965 the cows, weighing about 450 kilograms each, have received as much as 650 grams of urea per day.

To promote rumination, various methods have been used. Rumination in a lactating test cow has been markedly stimulated through the feeding of about half a kilogram of polyethylene pellets per day. In order to increase the secretion of saliva the test cows have been allowed to chew rather hard rubber tubing; this is placed above the feeding trough so that the saliva flows back into the mouth. The cows chewed the tubing very eagerly during the first years, when urea was used in the feed in smaller amounts than later on. At present they have lost to a great degree their interest in chewing the tubing. Eagerness to chew the tubing and thus promote the secretion of saliva may be connected with an inadequate intake of nitrogen.

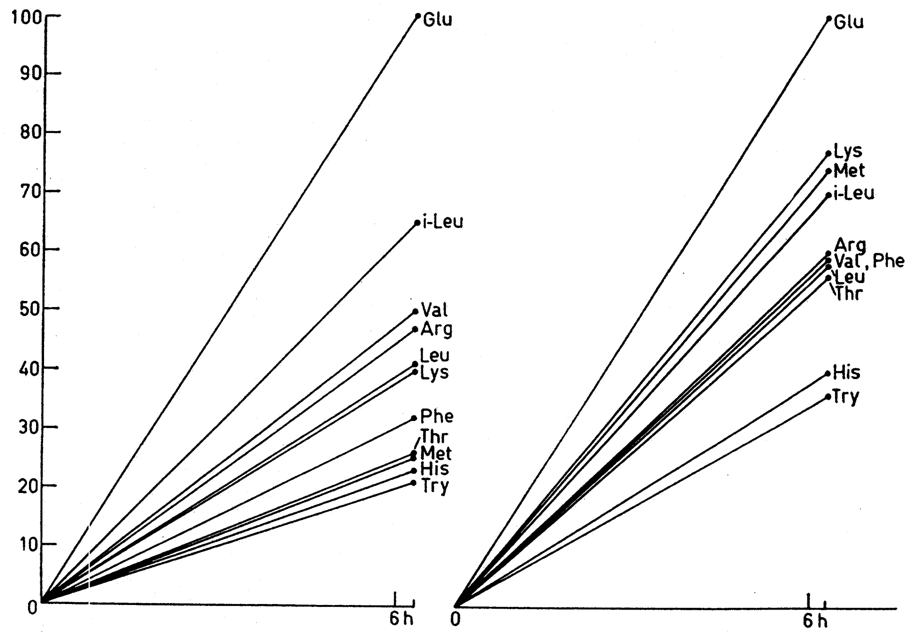


Fig. 1. Labeling of the essential amino acids of total milk protein 6.3 hours after the cow had been fed a single dose of ^{15}N-urea. The results are expressed as a percentage of the labeling of glutamic acid. At left, results of a feeding experiment with a cow on normal feed (17 March 1966); at right, results of a feeding experiment with a test cow (20 October 1962) 6 months after the start of the experimental feeding. Histidine and tryptophan have the lowest labeling in both experiments, but the increase in their labeling in the cow on the experimental feed is remarkable. [Determinations by M. Kreula and T. Moisio]

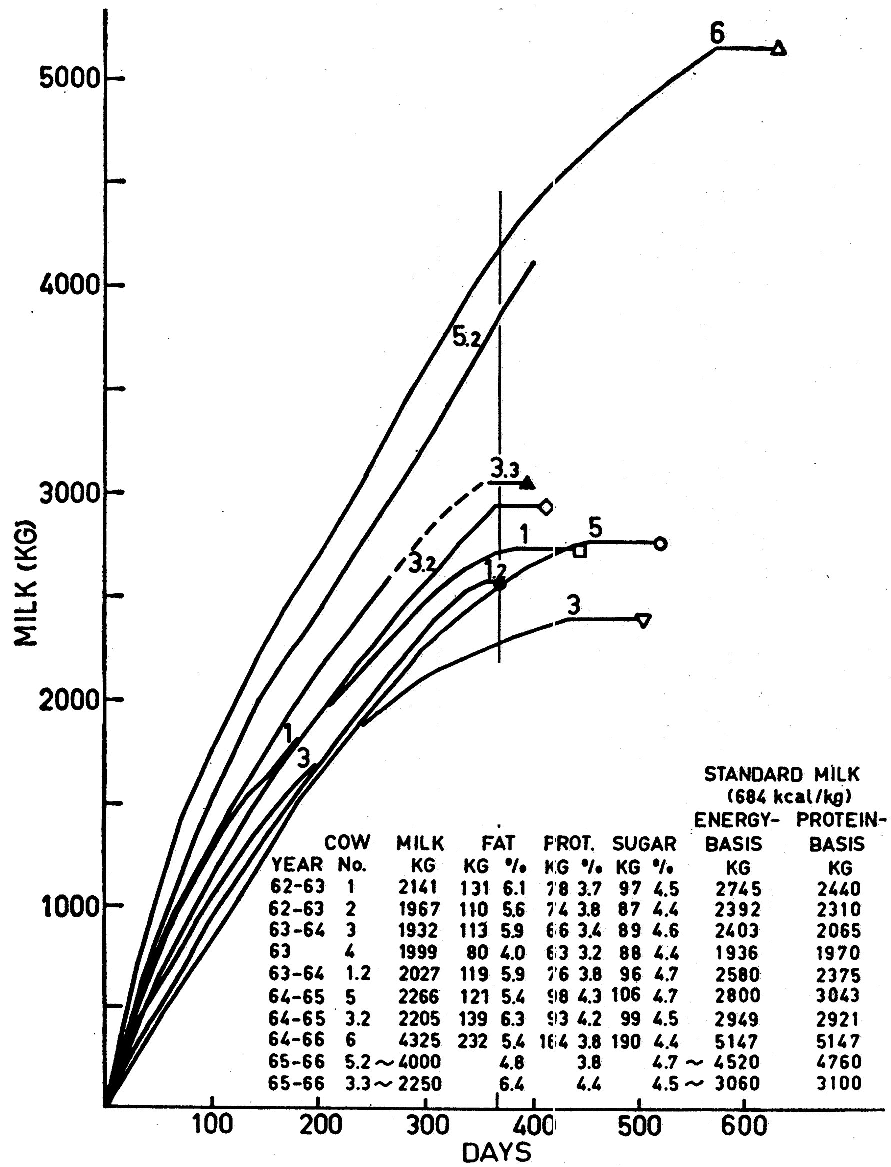
The digestibility of the urea and the nitrogen balance were determined several times, through the method of collecting feces and urine separately and the method of feeding chromic oxide (Cr_2O_3) as the marker and collecting feces and urine together. In addition, when the digestibility of nitrogen alone was to be determined, the chromic oxide method was used. Raising the urea content in the feed enhanced the digestion coefficient for urea nitrogen from 63 ± 0.97 to 70 ± 1.2 when the nitrogen consumed (in grams of nitrogen per kilogram of organic food) was raised from about 18 to 23. The increase in the digestion coefficients when more urea was fed was statistically highly significant. The nitrogen balance (in grams per day) averaged about + 13 when the cow was on a higher urea ration.

The results of studies to determine the dependence of the digestion coefficient on the amount of nitrogen fed agree with the observation of Raleigh *et al.* (*8*), who found in experiments with steer calves, that the digestibility of nitrogen significantly increased with each increase in the nitrogen content of the diet fed, regardless of the source of the supplement. In their experiments urea, used as the sole supplement to raise the nitrogen content of a low-quality meadow-hay roughage from 8.8 to 19.2 grams of nitrogen per kilogram of organic substance, was found to be highly toxic.

The raising of the urea content of the diet greatly increased the milk yield. It also greatly reduced or totally removed a probable symptom of nitrogen deficiency which was observed when the feed with low nitrogen content was used: the continuous thinning or loss of the hairy coat of the fore part of the cows' legs, especially that of the hind legs, about 2 months after calving, and the rapid regrowth when the daily milk yield had decreased to about 7 kilograms.

The estrus of the test cows has been regular and easy to observe. However, many of the cows have required several services. Each of the test cows has become pregnant, and two of the test cows, Eiru and Aino, have already calved three times. It is possible that at least one of the reasons for the requirement of several services is the deficiency of vitamin E. The test cows were not given vitamin E preparations until the end of 1964. During 1965 they were fed α-tocopherol daily (30 milligrams per cow); since January 1966 the amount has been increased to 330 to 500 milligrams. The vitamin E in the plant oil fed is probably not sufficient to satisfy the need of the milking cow for this vitamin, especially because polyunsaturated fatty acids enhance the requirement for vitamin E.

Altogether six cows have been fed the experimental feed (Fig. 2). The first four test cows (all Ayrshire breed) were chosen as representatives of cows with a relatively low milk yield (2500 to 3500 kilograms of milk and 104 to 154 kilograms of fat per year) on normal feed because the protein requirement of such cows can more easily be satisfied. Only cow No. 6 (Metta) had produced on normal feed as much as 4000 to 6000 kilograms of milk and 167 to 244 kilograms of fat per year. The feed adaptation of test cow No. 5 (Jairu) was started when she was a heifer, 2 months before her first calving.



YEAR	COW No.	MILK KG	FAT KG	FAT %	PROT. KG	PROT. %	SUGAR KG	SUGAR %	STANDARD MILK (684 kcal/kg) ENERGY-BASIS KG	STANDARD MILK (684 kcal/kg) PROTEIN-BASIS KG
62-63	1	2141	131	6.1	78	3.7	97	4.5	2745	2440
62-63	2	1967	110	5.6	74	3.8	87	4.4	2392	2310
63-64	3	1932	113	5.9	66	3.4	89	4.6	2403	2065
63	4	1999	80	4.0	63	3.2	88	4.4	1936	1970
63-64	1.2	2027	119	5.9	76	3.8	96	4.7	2580	2375
64-65	5	2266	121	5.4	98	4.3	106	4.7	2800	3043
64-65	3.2	2205	139	6.3	93	4.2	99	4.5	2949	2921
64-66	6	4325	232	5.4	164	3.8	190	4.4	5147	5147
65-66	5.2	~4000		4.8		3.8		4.7	~4520	4760
65-66	3.3	~2250		6.4		4.4		4.5	~3060	3100

Fig. 2. Milk production of test cows on experimental feed. The milk yield was calculated both on the basis of energy (standard milk, 684 kilocalories per kilogram of milk) and of protein (standard milk, 3.2 percent protein). The large numbers on the curves refer to the test cows, the small numbers to the calving times during the experimental feeding (for example, 3 = cow No. 3, first calving; 3.2 = cow No. 3, second calving); the end points of the curves represent the calving days. The annual milk yields may be seen from the curves. [No curves are shown for cows No. 2 and 4 as both cows died during the test period (7).] The feeding experiments were performed in cooperation with M. Lampila (1961–63) and T. Ettala (1963–).

Figure 2 shows the annual yield of milk, milk fat, milk protein, and milk sugar for each of the test cows on the experimental feed for various lactation periods (*9*). It also shows the milk yields per year and the length of lactation periods. The figures in column 10 show the milk yield calculated on an energy basis; those in column 11 show the milk yield calculated on the basis of the protein content. Values of 9 kilocalories for fat and 4 kilocalories for protein and sugar per gram were used in the calculations.

The curves of Fig. 2 show how greatly the milk yield has risen since the amount of urea in the feed was increased in 1964. So far, the highest production of standard milk per year has been 4217 kilograms; the highest production for a prolonged lactation period (18 months) has been 5147 kilograms (cow No. 6, Metta).

The weight losses of the test cows after calving were slight if the daily milk production did not exceed 15 kilograms. When the production reached 17 kilograms the weight loss over the following 6 weeks was about 30 kilograms; after this the weight remained unchanged for many months, and then rose. The initial loss of weight was still greater if the production of milk was 20 kilograms or more, but the weight changes thereafter were similar to those of the cows with lower initial milk production. The test cows have not eaten more than 11 kilograms of dry substance per day (about 10 Scandinavian feed units), hence insufficient energy intake leads to consumption of the body reserves during a period of high production of milk. Test cow Metta, which has given the highest daily yield (24.1 kilograms of standard milk), has, in spite of that, remained in good condition (Fig. 3).

The food value of the carbohydrates used was estimated by calculating the feed units on the basis of the amounts of feed consumed and the amount of milk produced. The calculations were made on data accumulated over a selected long period when the weight of the cow remained practically unchanged, so that weight increase or decrease could be omitted from the calculations. Calculations made on the basis of feed consumption and milk yield of test cow Jairu are as follows.

The cow calved for the second time on 19 May 1965. The calculations were made for a period of 77 days: 1 July through 15 September 1965. Weight on 30 June was 445 kilograms; on 31 August, 445 kilograms. Total milk production was 864 kilograms (5.1 percent fat, 3.8 percent protein, 4.8 percent sugar), equivalent to 1010 kilograms of standard milk, or to a daily average milk production of 13.1 kilograms of standard milk. During the 77-day period the cow was fed 670 kilograms of organic substance, consisting of 660 kilograms of carbohydrates (52.2 percent starch, 25.2 percent α-cellulose, 22.6 percent sugar) and 10 kilograms of fat.

With values of 3.5 feed units per day as the requirement for the cow's maintenance and 0.37 feed unit the requirement for production of 1 kilogram of standard milk, it was calculated that 1.05 kilograms of carbohydrates correspond to 1 feed unit. In all, 16.8 kilograms (99.6 percent of total nitrogen in the feed) of urea nitrogen and ammonium nitrogen was fed, corresponding to 11.8 kilograms of digestible nitrogen, or to 18.3 grams of digestible nitrogen per feed unit. This corresponds to 114 grams of digestible protein per feed unit. The total amount of nitrogen per kilogram of organic substance was 25.1 gram.

On the basis of calculations of this kind for three test cows, including Jairu, it has been established that 1.05 to 1.13 kilograms of carbohydrates correspond to one feed unit. Cellulose reduced the digestibility of the total carbohydrates, perhaps because the cellulose powder in the feed briquettes and in the cellulose-rich paste passes too quickly through the rumen to be digested. Actually, the feces of the test cows contain mainly fine-graded cellulose. In addition, it has been found that vigorously bleached sulfite cellulose is generally less digestible than unbleached (*10*). For the α-cellulose powder used, we found variable values, between 40 and 60, for the digestion coefficient.

Data on the milk production and the intake of carbohydrates, urea nitrogen (ammonium nitrogen included), and vegetable oils of test cow Metta are given in Fig. 4.

Microbial Flora and Protein Synthesis in the Rumen

The observations on the rapid utilization of ammonia in the rumen of the cows adapted to the test feed could be explained only by supposing that the microbial flora of the rumen content had been effectively adapted to the utilization of ammonium nitrogen. Investigations in this laboratory concerning the microbial flora of the rumen have shown that the protozoa in the rumen of the adapted cows have decreased enormously in number or have entirely disappeared, while the number of bacteria has risen remarkably. (The average number of bacteria counted microscopically in the rumen of two normally fed cows was 25×10^8 per milliliter, and the number for test cow Jairu was 12×10^{10} per milliliter.)

So far, only some preliminary studies of the bacterial flora of the rumen contents of the test cows have been made in this laboratory. It is extremely difficult to get a clear picture of the total microbial flora of the rumen and of its function, as the numerous investigations published up till now have revealed. It is probable that the bacterial strains which grow well with NH_4^+ without amino acids, vitamins, or other specific growth factors are the most important in the bacterial flora of the rumen of the test cows. Some strains of this type have already been isolated from the rumen of the test cows. They build up their own cell substance from ammonia and simple carbohydrates. Investigations on some bacterial strains isolated show again the capability of many rumen bacteria to use several different carbon sources for their energy metabolism when living in symbiosis. Regarding the symbiotic growth, I refer to the fundamental work of Nurmikko in this laboratory on the lactic acid bacteria (*11*). He showed conclusively that different strains of lactic acid bacteria can feed one another with different growth factors when they are grown in the same medium, because the factors synthesized in the bacterial cells are partly excreted into the medium. This means that in mixed cultures different strains can grow in a medium that is simpler than that needed by the same strains in pure cultures. The symbiotic growth is probably general in microbial associations, and the microbial flora of the rumen contents is one of the most complicated examples of this type of system. Although in the rumen of the test cows the bacteria with simple nutrient requirements form the body of the bacterial flora, strains which need specific factors for their growth are probably important for the effective function of the rumen. Bryant and Robinson (*12*) have recently studied the nutritional characteristics of ruminal bacteria of cows on normal feed, and they have found, among other things, that about 80 percent of 89 freshly isolated strains grew with NH_4^+.

In the amino acid composition of the hydrolyzate of the total protein of

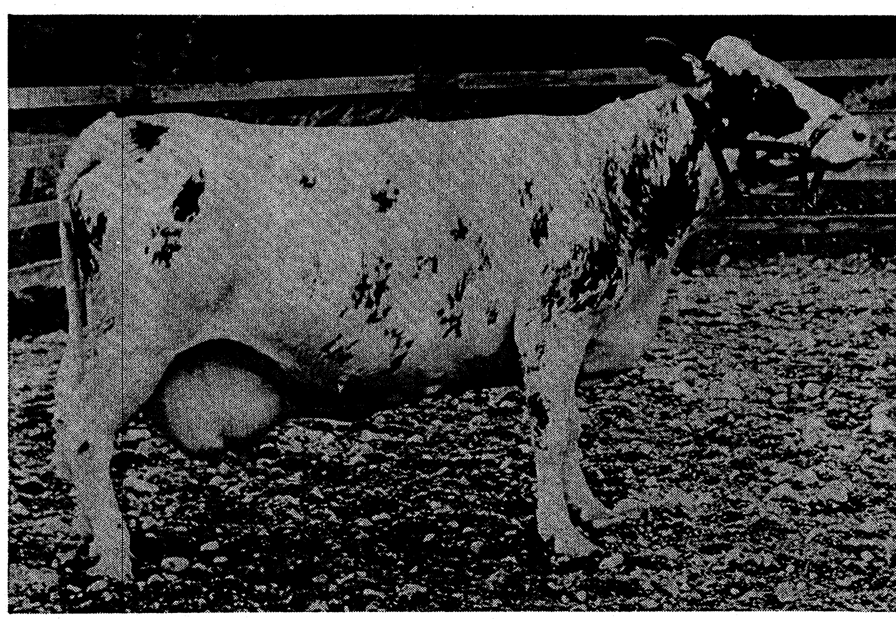


Fig. 3. Test cow Metta after being on test feed 370 days from calving.

the rumen contents of test cows and of control cows on normal feed, systematic differences could be found only regarding α,ϵ-diaminopimelic acid, a characteristic constituent of the cell wall of many bacteria (*13*). It was regularly present in much higher amounts in the hydrolyzate of the rumen protein of test cows (1.5 ± 0.1 percent of total amino acids) than in that of normally fed cows (0.4 ± 0.1 percent). There was some difference in histidine values for lactating test cows (1.1 ± 0.3 percent) and normally fed cows (0.7 ± 0.1 percent), but the difference was not statistically significant. The tryptophan content was not determined. It should be emphasized that the amino acid composition of the rumen protein is also dependent on the time of the sampling. This makes the drawing of conclusions about protein synthesis on the basis of rumen samples more difficult. Ellis *et al.* (*14*) have concluded, on the basis of their studies of the amino acid composition of the rumen contents of lambs, that the microbial synthesis of certain essential amino acids—according to them, methionine and tryptophan—is inadequate for the intensive protein synthesis needed f[...]
good growth.

Studies on the composition of tl
nitrogenous compounds of the rum(
contents have shown that the ar
monia concentration in the rumen (
cows on normal feed (protein-rich goc
silage, crushed oat, and hay) is high
(11.1 to 26.6 milligrams per 100 mil
liters; average, 15.7 ± 1.5) than tl
concentration for test cows (0.7
10.6 milligrams per 100 milliliter
average, 4.8 ± 1.3). The determin
tions were made 1 hour after tl
samples of rumen contents were take
In the same samples, also, total nitr
gen in nitrogen fractions soluble in 7
percent ethanol (free amino acid
"peptides," ammonia, urea), protein r
trogen, and volatile fatty acids we
determined. Protein nitrogen was ca
culated on the basis of the amino aci
formed in acid hydrolysis of the m
terial insoluble in 70-percent ethanc
There was a striking difference b
tween the protein content of the rum(
samples of the test cows and of no
mally fed cows. The protein nitrog(
of the rumen samples of test co
Jairu was 67.6 ± 2.3 percent of tot
nitrogen; the percentages for the tw
normally fed cows were 26.8 ± 3
and 26.2 ± 2.4, respectively. On tl
other hand, the nitrogen compoun
soluble in 70-percent ethanol occurr(
in much lower concentrations in tl
rumen samples of the test cows th
in the samples of the cows on norm
feed (Table 1). The results are
agreement with the findings, describ(
in this article, of highly enhanc(
synthesis of bacterial protein in tl
rumen of the test cows. The concentr
tion of total volatile fatty acids in tl
rumen contents is also seen from tl
data of Table 1. There was a relative
smaller amount of acetic acid in tl
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The molar ratio of acetic acid to pr
pionic acid for test cow Jairu w
1.47 ± 0.06, and for three contr
cows on normal feed (good silage, ha
crushed oat), 3.32 ± 0.33. The amou
of butyric acid was similar in the tw
cases; the concentrations of *n*- and
valeric acids were low and variable f(
the test cow and the normally f(
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The composition of the nitrogenou
compounds of the feces of the test cov
has been closely studied in this labor
tory. In experiments with rats, M
Naught *et al.* (*15*) found the digestic

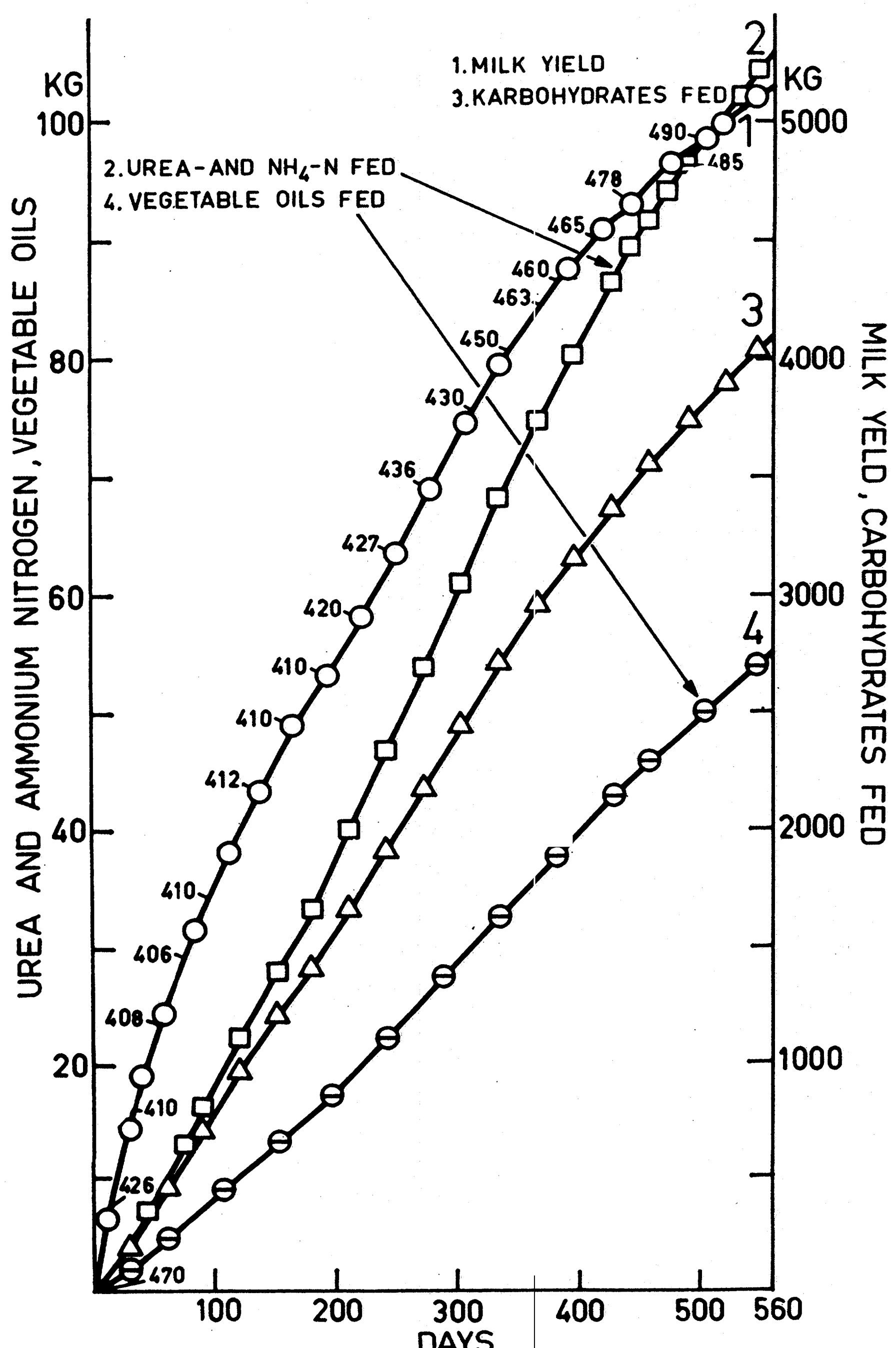

Fig. 4. The production of milk and the intake of carbohydrates, urea nitrogen (NH_4–N included), and vegetable oils for test cow Metta.

coefficient of the protein in a bacterial fraction grown in strained rumen liquor with urea and carbohydrate supplement to be 74. If this coefficient is the same in ruminants, the main part of the nitrogen of the feces of the test cows should be indigestible bacterial protein. In fact, among the normal amino acids which are found in the hydrolyzate of the protein fraction of the feces of the test cows, α,ϵ-diaminopimelic acid is present in much higher amounts than in the same fraction from cows on normal feed (the averages are 2.7 ± 0.49 and 0.6 ± 0.23 percent of total amino acids of the hydrolyzate, respectively). This is in agreement with findings for the amino acid composition of the proteins of the rumen contents. Although not all rumen bacteria contain diaminopimelic acid in their cell wall "proteins" (*16*), the high content of this amino acid in the protein fraction of feces strongly supports the implication of other results, presented above, that bacterial protein synthesis is greatly enhanced in the rumen of the test cows.

Table 1. Analyses of rumen contents of a test cow and a normally fed cow. [T. Ettala]

Dry matter (%)	Total N (mg/100 ml)	Protein N (% of total N)	N soluble in 70% ethanol (% of total N)	N-fractions soluble in 70% ethanol: NH_4-N (% of total N)	Free amino acid N (% of total N)	"Peptide N" (% of total N)	Volatile fatty acids (mmole/100 ml)
			Test cow Jairu				
6.58±1.3	153.9±34	67.6±2.3	10.0±2.6	1.9±0.47	3.1±0.99	5.5±2.1	9.4±1.3
			Normally fed cow				
2.25±0.37	85.4±10	26.8±3.0	43.6±8.8	16.2±1.0	10.9±3.2	11.7±4.6	7.4±1.7

Composition of Nitrogenous Compounds in Blood of Test Cows

Because the mammary gland receives the raw material for the synthesis of different components of milk from the blood, knowledge of the composition of the blood of the test cows is important. A great number of analyses have been made of the whole blood and plasma of cows on test feed and, for comparison, of cows on normal feed. The blood samples (about 150 milliliters each) were taken from the jugular vein. Of the nitrogenous compounds, the free amino acids of the plasma and the whole blood, the amino acids of the whole-blood proteins, the glutathione and ammonia of the whole blood, the urea of the plasma, and the total nitrogen of the plasma and the whole blood were determined from the blood samples over a long period (see *17*). The most important results are given below.

The free amino acids of the blood form only a very small part of the nitrogenous compounds of the blood, but they are decisively important for the formation of the proteins of milk in the mammary gland. The latest studies (*18*) have shown that the proteins of milk are mainly formed from the free amino acids found in the blood. Therefore in our laboratory attention has been directed especially toward the amounts of free amino acids in the blood plasma. The concentrations of most of the free amino acids, particularly the essential amino acids, in the plasma and the whole blood of the lactating test cows were lower than those in plasma and blood of the control cows on normal feed. The relative decrease in the concentration of free histidine in plasma was greater, in cows on the test feed, than the decrease for any other amino acid. The histidine content decreased some weeks after calving, remained low for several months, and rose again, to some extent, while the milk production decreased. For instance, the histidine content of the blood plasma of test cow Jairu 5 weeks after calving was 0.93 milligram per 100 milliliters, but 6 weeks later it was 0.23 milligram. At its lowest level, the free histidine content of the plasma was about 20 percent of the corresponding values for the normally fed cows. The free methionine content of the plasma of test cows was about 67 percent of the values for normally fed cows, but, because the concentration of methionine in the plasma of normally fed cows is low, its decrease in cows on test feed may be of great significance. The sum of methionine and cystine for the test cows is, however, not very low.

In Fig. 5 the values for the contents of some free amino acids in the plasma of the lactating test cows and normally fed cows are given. Low concentrations of free amino acids are found in the plasma of the lactating test cows; the concentrations of histidine and many

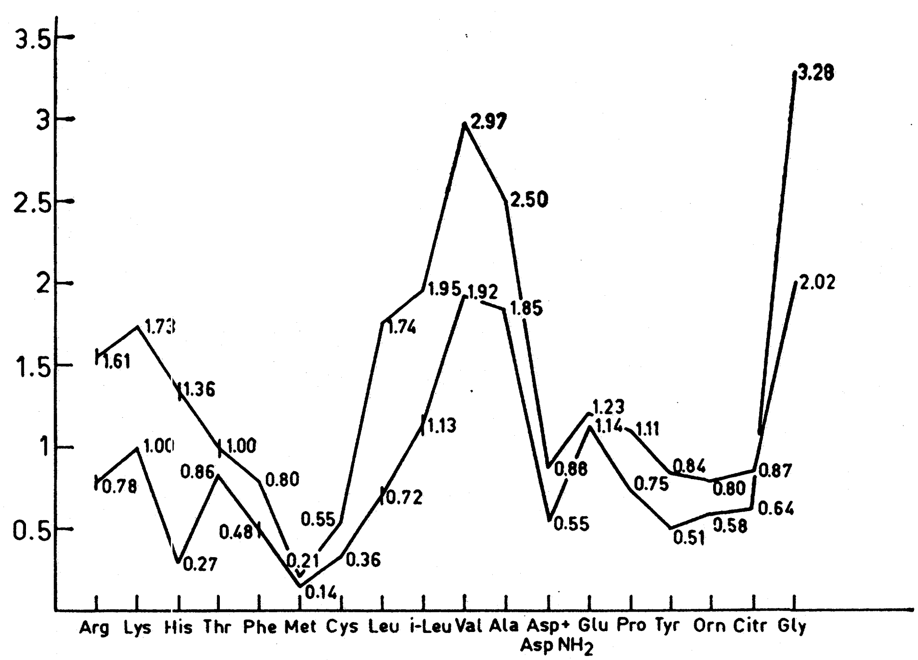

Fig. 5. The contents of some free amino acids in the blood plasma of the lactating test cows (values on lower curve, except for glycine) and normally fed cows (values on upper curve, except for glycine). Glycine is the only amino acid which is present in higher concentration in the plasma of test cows than in that of normally fed cows. The numbers on the curves signify free amino acids, in milligrams per 100 milliliters of plasma. The fall of the free histidine to 20 percent of normal values is noteworthy.

other amino acids in the plasma of dry test cows and of heifers is much higher. The concentration of free glycine in the plasma was higher for the lactating test cows than for the control cows and the dry test cows. The concentrations of plasma urea and plasma total nitrogen were similar for the test cows and the control cows. So, also, were the values for total volatile fatty acids. On the chromatograms of the free amino acids of the blood and plasma of the test cows and the control cows on normal feed, 17 ninhydrin-positive compounds could be consistently detected, besides the 24 common substances.

The blood concentration of ammonia was determined according to the Conway method (*19*). Immediately after sampling, which always was carried out about 6 hours after the start of the feeding, no ammonia could be found in the blood of either the test cows or two normally fed cows, but, if the determinations were performed 2 hours after sampling, small amounts of ammonia were found. There is surely an absorption of ammonia into the blood across the rumen wall, as many authors have shown, but, in our experiments, when the samples were taken many hours after the feeding the accumulation in blood from the jugular vein could not be observed. In experiments with normally fed goats given doses of urea intraruminally, Abdel-Rahman (*20*) found a gradual increase in the urea concentration in plasma over a period of 2 hours; however, the ammonia and glutamine concentrations rose suddenly and temporarily half an hour to 1 hour after the administration of urea. It is to be emphasized, however, that the animals had not been adapted to urea.

The observations made in this laboratory of the low amount of ammonium nitrogen in the rumen of the test cows show the great ability of the adapted ruminal flora to utilize ammonium nitrogen. This may explain why ammonia is not accumulated in disturbing amounts in the blood in spite of the large amounts of urea fed. Whether the ability of various organs of the test cows—for example, the liver—to utilize ammonium nitrogen has increased is not known. Holzschuh and Wetterau (*21*) have recently studied the effect of urea administered to cows by various means on the ammonia concentration of the blood. After a slight adaptation of the cow to urea, a diminution of maximum concentration of ammonia was observed. Holzschuh and his co-workers assume that this was due more to an acceleration of biochemical reactions in the liver than to a more rapid binding of ammonia in the rumen.

The total nitrogen in the blood of the lactating test cows is lower than that for normally fed cows, due mainly to differences in the hemoglobin content. When the test cows are dry, the hemoglobin content of their blood is normal (13 to 14 grams per 100 milliliters of blood), but some weeks after calving the hemoglobin content decreases. In late lactation, when the milk production is low, the hemoglobin content rises gradually; it reaches a maximum when the cows are dry. For instance, the blood hemoglobin of test cow Jairu was 13.8 grams per 100 milliliters of blood when the cow was dry. One month after calving the value was 12.9 grams; 3 months after calving, 10.4 grams; 5 months after calving, 10.3 grams; and 11 months after calving (with milk production still 8 kilograms per day), 13.7 grams. It is still not known what the reason is for the reduction of the hemoglobin level of a milk-giving cow on test feed. Since the amount of free histidine in the blood of the test cows is very low, and since it appears, from observations presented above, that histidine may form a bottleneck in protein synthesis in cows on the test feed, it may be that the primary reason for this reduction in hemoglobin is the deficiency of histidine during the post-calving period, when the protein requirement is greatly increased because of milk production. As is known, the histidine content of hemoglobin is high, more than 8 percent. Also other factors can be suspected—for example, a possible deficiency of vitamin B_{12}. The finding that the concentration of vitamin B_{12} in the test milk is similar to that in normal milk neither supports nor refutes this hypothesis because the secretion of vitamin B_{12} may occur at the expense of the organism.

The concentrations of most of the free amino acids and of the unknown compounds were higher in the whole blood than in the plasma (for example, the concentration of histidine in the plasma of the test cows was 0.27 $\pm$ 0.02 milligram per 100 milliliters of plasma, whereas for cows on normal feed the value was 1.36 $\pm$ 0.09 milligrams; the concentration in the whole blood of the test cows was 0.62 $\pm$ 0.05 milligram per 100 milliliters; for cows on normal feed the value was 1.85 $\pm$ 0.09 milligrams). The concentration of the glutathione was generally higher in the blood of the control cows than in that of the test cows (the respective values are 41.0 $\pm$ 1.2 and 23.0 $\pm$ 1.4 milligrams per 100 milliliters of blood).

The amino acid composition of the whole-blood protein was generally similar in the test cows and the normally fed control cows. In electrophoretic studies, carried out in this laboratory, on the serum proteins from several blood samples of the test and control cows, only slight differences in the various protein fractions in samples from individual cows could be observed, and the differences in the results for test and control cows were slight.

For the milk-giving test cows, the total cholesterol of blood plasma has averaged 69 $\pm$ 3 milligrams per 100 milliliters of plasma, and for two cows on normal feed the average is 210 $\pm$ 23 milligrams. Esterified cholesterol accounted for an average of 87 percent of the total plasma cholesterol in both the test cows and the normally fed cows. The values for total plasma lipids of the test cows and the normally fed cows, respectively, were 158 $\pm$ 8 and 522 $\pm$ 64 milligrams per 100 milliliters of plasma. By contrast, plasma concentrations of total volatile fatty acids were almost the same in the test cows and the normally fed cows.

Composition of the Test Milk

The composition of the test milk, when the quantitatively most important compounds are taken into consideration, corresponds to the composition of normal fat-rich milk with respect to fat and protein. The average fat content, with the exception of the milk from one test cow, has varied from 4.8 to 6.4 percent, while the fat content of the milk of the same cows before transfer to the test feed ranged from 4.2 to 4.8 percent. The rise in the fat content of the milk of the cows on the test feed may be in part a result of the somewhat decreased milk yield, but in cows on this feed there is a tendency toward increased fat production. When the content of urea in the feed was raised the fat content remained about the same but the protein content rose remarkably. It thus seems that the amount of urea fed has a positive influence on the protein content of the test milk but not on the fat content. The sugar content of the test milk (4.4 to 4.7 percent) has been somewhat lower than that of normal milk. Even such low sugar values as 4.1 percent have been observed in milk samples with a protein content of 5 percent or more.

The composition of the nitrogenous substances, especially the proteins, of the test milk has received our special attention. At the beginning it was found that the ratio of total nitrogen to residual nitrogen (nitrogen remaining after acid precipitation of casein) was very similar for test milk and normal milk. A great number of analyses of milk from test cows gave, for the ratio of total nitrogen to residual nitrogen, the average value 4.49 ± 0.13; the ratio for milk from normally fed cows was 4.76 ± 0.33. The percentage of nonprotein nitrogen in the test milk was 4.9 ± 0.23 and in normal milk from different farms, 4.2 ± 0.35. In test milk the percentage of ammonium nitrogen and urea nitrogen in the total nitrogen was 1.1 ± 0.1; for normal milk of six cows the average percentage was 2.0 ± 0.1; for mixed milk from five farms the percentage was 1.6 ± 0.07. The amino acid composition of casein and total protein has been estimated, after acid hydrolysis, in hundreds of milk samples, though the tryptophan content has been determined in a few samples only. The values for the amino acids of the total protein and casein of the test milk are so close to those of milk produced on normal feed that no conclusive differences could be demonstrated (*7*).

Nor has the fractionation of the proteins shown any differences between the test milk and normal milk (*22*). Both the direct fractionation of the proteins of milk on a diethylaminoethyl-cellulose column and the fractionation of casein and the serum proteins of milk by means of gel electrophoresis have shown the similarity between the test milk and normal milk. Small differences in some smaller protein fractions of the milk of different cows have been observed, but they seem to be of an individual character. No conclusive differences, between test milk and normal milk, could be found in the activities of the milk enzymes studied when allowance had been made for the appreciable individual variations. So far, peroxidase, xanthineoxidase, aldolase, amylase, alkaline phosphatase, and lipase activities have been determined.

These results show how extraordinarily effective the protein synthesis in the mammary gland is. In spite of the low concentration of some free essential amino acids in the blood of the test cows, the mammary gland is capable of synthesizing a surprising amount of milk protein, the numerous components of which have a normal composition. The high protein content and low urea and ammonium content of test milk are particularly remarkable.

In a comparsion of test milk and normal milk, the only major constituent showing large differences in composition is the fat. As is well known, the composition of the milk fat is greatly influenced by the nature and amount of the fatty acids in the feed of the cow. Since there is practically no fat in our test feed, apart from the vegetable oils added, it has beeen possible, in our feeding experiments, to make significant observations on the biosynthesis of the fatty acids. In Tables 2 and 3 some results of the fatty acid analyses of the test-milk fat may be seen.

These results show certain relationships, as follows.

1) The palmitic acid content of the fat of the test milk was exceptionally high—almost 50 percent of the total fatty acids—when the daily amount of oil fed was as low as 37 grams per cow; the total production of fat of test cow Eiru was 428 grams and of test cow Jairu, 358 grams per day. When the amount of oil fed was doubled, the pal-

Table 2. Amounts of fat in diet, milk production, and milk-fat production for test cow Jairu (calved 24 December 1963 and 19 May 1965) and for cows on normal feed, for three different feeding periods. See Table 3 for associated data on individual fatty acids.

Feeding period	Fat in diet (g/day)			Milk production (kg/day)	Milk-fat production (kg/day)
	Olive oil	Maize oil	Linseed oil		
		Jairu			
11 Jan.–20 Feb. 1964	0.0	18.4	18.6	7.61	0.358
11 July–20 Sept. 1965	36.6	46.0	46.5	10.66	.559
		Cows on normal feed			
25 Sept. 1963–15 May 1964				~ 10	~ .450

Table 3. Individual fatty acids in the milk fat of test cow Jairu and of control cows for the three feeding periods of Table 2.

No. of carbon atoms in individual fatty acids*	Individual-fatty-acid content (% of total fatty acids) in milk fat of test cow Jairu		Individual-fatty-acid content (% of total fatty acids) in milk fat of control cows, 25 Sept. 1963—15 May 1964
	20 Feb. 1964 11 Jan.–	11 July–20 Sept. 1965	
4	2.63	3.05	3.05
5	0.13	0.05	< 0.02
6	1.90	1.73	1.76
7	0.12	0.05	< 0.02
8	1.08	1.12	1.02
9	0.12	0.06	< 0.02
10	2.38	2.73	2.13
10:1	0.44	0.40	0.25
11	0.22	0.14	< 0.02
12	3.64	3.74	2.65
12:1	0.19	0.18	0.08
13 br	0.15	0.17	0.03
13	0.51	0.26	0.04
14 br	0.32	0.21	0.09
14	10.17	10.95	9.87
14:1	2.63	1.95	1.01
15 br	0.52	0.82	0.50
15	4.88	2.70	0.90
16 br	0.48	0.33	0.19
16	48.16	38.90	28.41
16:1	4.29	4.22	2.03
17 br	0.94	0.96	0.58
17	1.57	0.90	0.46
17:1	1.13	0.77	0.39
18	0.98	2.58	12.29
18:1	8.35	19.14	29.37
18:2	1.40	1.15	1.79
18:3	0.67	0.74	1.11

* Numerals 1, 2, and 3 following a colon indicate the number of double bonds; br indicates branched-chain fatty acid.

mitic acid content decreased to about 40 percent of the total fatty acids. Raising the amount of oil to 129 grams decreased further the proportion of palmitic acid, but it was still high.

2) The amount of oleic acid was correspondingly very low—about 10 percent of the total fatty acids when the smallest amount of oil was used in the feed. The percentage was doubled when the greatest amount of oil was used, but it was still considerably lower than that of milk fat produced on normal feed. The amount of stearic acid was especially low, rising only a little after the amount of oil in the feed was increased.

3) The contents of unsaturated fatty acids which occur in lesser amounts —especially of C_{16} and C_{14} acids —were higher in the fat of the test milk than in the fat of normal milk.

4) The amount of branched-chain fatty acids was also somewhat increased in the fat of the test milk.

On the basis of these findings on the production of milk fat and the composition of its fatty acids, the hypothesis seems justified that the fatty acids of the milk fat of the test cows on low fat rations are synthesized mainly in the mammary gland, and that the synthesis of the fatty acids containing more than 16 carbon atoms is weak. According to Riis (*23*) there is evidence that, with normal feed, the synthesis within the mammary gland accounts for about half the fat in cow's milk, the other half being derived directly from the blood. The low content of C_{18} acids in the fat of test milk, together with the lowered lipid content of the blood of the test cows, suggests that a vigorous synthesis of fatty acids and fat occurs in the mammary gland and that the derivation from the blood is low. The generally accepted opinion that a high ratio of propionic acid to acetic acid in the rumen causes a fall in the percentage of milk fat does not hold true for test milk. The reason for the high fat content of this milk is not known. The rather low sugar content of the test milk may be the limiting factor of the milk production. Propionate may cause the rise in the content of odd-numbered fatty acids of milk fat, and the bacterial flora in the rumen may cause the rise in the content of branched-chain fatty acids (see Table 3). The increase of C_{16}, C_{14}, C_{12}, and C_{10} acids with one double bond in the fat of test milk is noticeable. Therefore, in spite of the low content of oleic acid in the fat of the test milk, the fat has a relatively high iodine number, usually from 28 to 32 when the highest amount of fat is fed. The especially low content of stearic acid suggests that the hydrogenation of the double bond in the rumen is lower in cows on the test feed than in cows on normal feed. This may be associated with the absence of protozoa in the rumen (*24*).

Using an extract of cow mammary gland, Ganguly (*25*) has shown the malonyl coenzyme A pathway in the synthesis of both short- and long-chain even-numbered fatty acids. The fatty acids formed were similar in chain-length distribution to those found in milk. Dils and Popjác (*26*) studied the synthesis of fatty acids from ^{14}C-labeled acetate by preparations of the mammary glands of lactating rats. On the basis of the distribution of ^{14}C among the fatty acids synthesized they concluded that, although stearic acid and oleic acid were synthesized, the concentrations were much lower than those found in milk. In agreement with this result, our feeding experiments suggest that, in vivo, the C_{18} acids are mainly derived from the blood, and the composition of milk fat is therefore dependent on the fatty acids in the feed.

The level of cholesterol in the test milk has been measured over a period of 2 years, samples from herds on pasture or on normal winter feed being taken as controls (*27*). The mean value for 93 samples of normal milk was 317 ± 4 milligrams of total cholesterol per 100 grams of milk fat, whereas the test-milk values were clearly higher, the mean being 409 ± 6 milligrams. Esterified cholesterol constituted about 6 percent of the total cholesterol in both test-milk and control milk. Patton and McCarthy (*28*) found evidence that cholesterol, in its intermediate esterified form, probably plays an important role in milk-fat synthesis in the mammary gland. In view of this finding, and also of the fact that the concentration of lipid in the blood of the test cows is low, the higher cholesterol values of test milk may indicate that the proportion of the total milk fat synthesized within the mammary gland of the test cow is higher than the proportion in cows on normal feed.

Preliminary studies on milk-lipoprotein material prepared from the buttermilk and butter serum of washed cream have been made. The yield of lipoprotein from both the test milk and normal milk was about 1 percent of the milk fat, in agreement with published values (*29*), and the protein remaining after lipid extraction comprised some 40 percent, by weight, of the lipoprotein of both test milk and normal milk. The nitrogen content of the protein of the lipoprotein of both milks was about 14.0 percent. The amino acid composition of this minor protein of the test milk agreed almost exactly with that of normal milk, in analogy with findings for the major milk proteins, the individual figures being similar to those reported by King (*29*). There appears to be a broad similarity between the amounts of the major fatty acid components of the total phospholipids of both test and control milk, but results so far are inconclusive.

Vitamin Content of Test Milk

The vitamin content of the test milk has been followed continuously since 1963, through determination of the vitamin-B complex by microbiological methods. Values obtained in 1963 showed that concentrations of thiamine, pyridoxine, folic acid, biotin, and B_{12} were much the same as in normal milk. The concentrations of riboflavin, nicotinic acid, and especially pantothenic acid were generally higher in the test milk than in normal milk. Values obtained in 1964 and 1965 were, in most respects, similar to the earlier values, but no statistically significant differences between test milk and normal milk with respect to the contents of riboflavin and nicotinic acid could be found (Table 4). To judge by the vitamin determinations, the biosynthesis of the vitamin-B complex brought about by the microorganisms in the rumen seems to be rapid enough to keep up a normal content of these vitamins in the test milk.

The finding of normal concentrations of vitamins of the B group in milk is not, of course, evidence that the cow itself is receiving enough of these vitamins. In the mammary gland the transfer of the vitamins to the milk might be so effective that the cow itself suffered from a lack of vitamins, but, in view of the fact that the experiment has lasted many years without evidence of such a lack, this can hardly be the case. The lowered concentration of hemoglobin in the blood of the milk-giving test cows might be connected with a deficiency of vitamin B_{12}, but there is no evidence in support of this hypothesis.

Porter (*30*) has reviewed the literature on vitamin synthesis in the rumen and has concluded that the adequacy of the synthesis of some vitamins of the B group in the rumen is questionable. Our observations made with a vitamin-free feed are in many respects enlightening and show at least that the bacterial flora developed through feed adaptation synthesizes effectively all vitamins of the B group.

Of the other vitamins, only A and D have been given as additives since the start of the feeding experiments. The concentrations of these vitamins in milk depend on the amount of vitamins fed. When 400 milligrams of α-tocopherol was fed daily to one of the test cows the milk contained vitamin E (20 to 40 micrograms per 100 milliliters). A slightly higher concentration is found in milk produced by cows on normal winter feed.

Table 4. Vitamin content of milk from test cows and from normally fed cows during years 1964 and 1965. [After M. Saarivirta]

Thiamin HCl (μg/100 ml)	Riboflavin (μg/100 ml)	Nicotinic acid (μg/100 ml)	Pyridoxine (μg/100 ml)	Folic acid* (μg/100 ml)	Biotin (μg/100 ml)	Calcium pantothenate (μg/100 ml)	B_{12} (mμg/100 ml)	Ascort acid (mg/100 m
				Milk from test cows				
45.7±1.8	306±18	167±4.9	57.8±5.2	2.91 ± 0.15	3.42±0.31	1120±55	455±46	2.78±0.
				Mixed milk from normally fed cows				
43.4±2.6	293±32	158±8.4	53.0±6.3	3.18±0.19	3.13±0.84	597±62	523±48	2.25±0.

* Values from 1963.

Flavor of the Test Milk

The flavor of the test milk has drawn particular attention in this laboratory. For comparison of the taste and aroma of test milk and normal milk it was necessary to keep the test feed as free of flavor substances as possible. In organoleptic tests the test milk was found to have a characteristic milk flavor without any off-flavors. Thus it appears that the basic flavor substances of milk are formed in the rumen and other organs of the cow.

This is somewhat surprising because there had been reason to expect that some of the different flavor substances found in the cow's normal feed would be transferred to the milk via the cow's body in amounts adequate to affect the flavor of the milk. Such a transfer has been observed in the case of some strong-smelling substances which cause characteristic off-flavors in milk. For example, the onion flavor of milk when the cows are on pasture contaminated with chive (*Allium schoenoprasum*) is well known. The substances causing this off-flavor are already relatively well known (different alkyl S_2 compounds, especially dipropyl S_2). A "burnt" or "scorched" flavor has been reported in the milk of dairy cattle that graze on pastures of Queensland and New Zealand which are infested with land cress (*Coronopus didymus*). This objectionable off-flavor is, according to the recent report of Park (*31*), produced by benzylthiocyanate, which, some years ago, was discovered in this laboratory (*32*) to be an enzymatic splitting product of benzyl mustard oil glucoside (glucotropeolin) in the crushed seeds of garden cress (*Lepidium sativum*) and in the green parts and seeds of *L. ruderale*. Benzylthiocyanate is also formed from the same glucoside in land cress (*31*). Off-flavors of other types are also transferred from the feed—for example, silage of poor quality—into the milk. Thus there is no doubt that the feed consumed by the cow could affect the flavor of milk adversely. One would thus expect that some fodder plants with a pleasant flavor would improve the taste and smell of milk. It is, however, very difficult to observe organoleptically such positive flavor effects.

The test milk produced on purified nutrients now offers new possibilities of elucidating the problem of the extent to which the normal flavor substances of milk are derived from the cow's feed and the extent to which they are formed in the cow's body in the absence of the large number of different and, to a great degree, unknown organic compounds contained in the plant material on which cows normally feed. Our studies in this field can be divided into three parts. (i) An analysis of the flavor substances of test milk and of milk produced on different normal feeds (good silage with pH not exceeding 4, hay of good quality, oats, beet, or pasture) is being made. (ii) Attempts are being made to identify the flavor compounds in some typical fodder plants commonly used in Finland. (iii) Experiments have been performed in which, by means of a plastic tube, pure chemical compounds of groups whose members have been identified as flavor compounds in fodder plants are passed into the rumen of a lactating cow (*33*). By gas-chromatographic analysis of tl milk, the degree of transfer of tl substances to the milk can be es mated.

By choosing substances of sufficien ly different retention times, it is poss ble to feed several compounds at tl same time for purposes of these stu ies. In our experiments we have fe a mixture of various homologous o ganic compounds (boiling range, 80° t 300°C). Most of the substances teste were transferred in trace amounts t the milk, the maximum concentratio being reached, in general, after hours. The compounds of higher molec ular weight tend, however, to reac maximum concentration somewhat late (after 4 hours). The concentration the rapidly decreases, so that after 1 to 14 hours—the normal interval be tween milkings—the concentration i only about 10 percent of the maximun (*33*). Very few flavor substances ar present in the fodder plants in sucl amounts that they could occur in mill in concentrations which would excee the flavor threshold. For instance, mos of the aliphatic alcohols, aldehydes ketones, and esters, at least those o molecular weight up to 150, are trans ferred from feed to milk via the cow's body in amounts too small to influence markedly the flavor of the milk. This explains why the milk flavor is not distinctly influenced by feed which does not have a pronounced off-flavor. It is true that some recent observations on the synergistic effects of different flavor compounds in subthreshold amounts (*34*) reduce the supposed usefulness of gas-chromatographic analysis of milk flavor. It is therefore all the more important that the test milk has made it possible to compare organoleptically normal milk produced on different types of feed with milk produced on purified diet.

It is not possible here to deal with the problem of milk flavor in greater detail. I will mention only that δ-lactones—probably the most important flavor substances of milk—occur in test milk, on the average, at least in the same concentrations as in normal milk. The concentrations of δ-lactones from C_6 to C_{10} are the same in test milk and normal milk, whereas the concentration of C_{12} lactone is higher in test milk. Methods for gas-chromatographic analysis and mass-spectrometric identification of δ-lactones and other flavor compounds in milk have been developed in this laboratory (*35*).

Summary and Conclusion

The synthesis of bacterial protein in the rumen of lactating cows fed on purified carbohydrates, with urea and ammonium salts as the sole sources of nitrogen, can be increased, through feed adaptation, to a level adequate not only for the maintenance of the cow but also for a relatively high milk production. The best annual milk yield per cow on the experimental feed has, so far, been 4217 kilograms, calculated as standard milk (684 kilocalories per kilogram of milk). The composition of the test milk is similar to that of normal fat- and protein-rich milk. Fractionation of casein and serum proteins of test milk and normal milk by different methods demonstrated the similarity of the proteins of the two milks. The normal or higher concentrations of the water-soluble vitamins in test milk show that the biosynthesis of these vitamins in the rumen is vigorous. The flavor of the two milks is very similar—proof that biosynthesis of the effective flavor compounds of milk occurs in the body of the cow. The only component of test milk whose composition, when small amounts of vegetable oil have been fed, has differed from that of normal milk is the fat.

The studies of milk production on the experimental feed have opened up new possibilities for investigating the biosynthesis of different milk components. The studies are also of practical importance: Since the vigorous biosynthesis of proteins from simple nitrogen compounds in the rumen of test-feed-adapted cows has been demonstrated, there are greater possibilities for studying the replacement of protein by urea. In countries where there are plenty of forests, part of the feed of cows can be made up of certain wood products—for instance, hemicellulose and cellulose of low quality. The new findings may also be of value in the dry areas of the globe, where milk would be of vital significance for improving the nutrition of the population.

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9. The following terms for milk are used in this article: *normal milk* is milk produced on normal feed (pasture, hay, silage, roots, concentrates, and so on), regardless of the composition of the milk; *test milk* is milk produced on a protein-free test feed, with urea and ammonium salts as the sole sources of nitrogen; *standard milk* is milk with energy yield of 684 kilocalories per kilogram of milk —for example, fat, 4.0 percent; protein, 3.2 percent; sugar, 4.9 percent. This is the standard of comparison on an energy basis. Milk with a protein content of 3.2 percent is the standard of comparison on a protein-content basis.
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36. This research was financed in part by a grant made by the U.S. Department of Agriculture, Agricultural Research Service.

Addition to the article in Science

The protein problem which has occupied so central a position in the feed of milking cattle, has lost this position through the investigations presented above. How the knowledge accumulated has to be applied in practice under various circumstances depends on carbohydrates available. In tropical areas sugar cane produces cheap carbohydrates which are suitable for cattle feed. The problem there is how to provide a feed rich in starch which is needed in a well-balanced diet containing plenty of urea. In the northern hemisphere it is the forests that produce raw material suitable for cattle feed when treated industrially. Under the circumstances that prevail in Finland it is advantageous to replace imported protein-rich concentrates with urea. From the point of view of the economic life of the whole country it would be most important to obtain a considerable part of the carbohydrates needed for milk and meat production from the by-products of the wood industry, which are now either discarded as waste into our water ways to pollute them or at best dried, at great expense, and burnt. If such a feeding system were successful, fields and meadows which are difficult to cultivate could be turned into very productive forests.

A practical feeding experiment using a feed which has included a considerable amount of wood hemicellulose, mostly hydrolyzed to sugar, and the so-called 0-fibre which is in part a waste product of the cellulose industry, was started at the beginning of January 1966. A heifer-cow, which subsequently calved at the end of February, was adapted to this test feed during a period of six weeks. The daily feed ration of the cow has included 7-20 kg potatoes, 3.5 kg dry pulp, 2.5 kg hemicellulose-sugar, 2 kg 0-fibre and 1.5 kg oats-straw. The daily dose of urea has been 440 g for the main part of the experimental period. Of the digestible nitrogen given daily to the cow only about 20 % has been protein nitrogen and about 80 % urea- and amide-nitrogen. About 600 g of a salt mixture have been given per day and of the vitamins A, D and

E vitamins. On such a feed the cow has produced, during a period of 280 days, a total of 4000 kg milk with an average of 4.1 % fat and 3.3 % protein. The annual production will probably reach about 4700 kg (Fig. 1). This practical feeding test in which potatoes and pulp have been included because of their low protein content shows that a considerable part, 30-40 %, of the feed of milking cattle can be made up of forestry products.

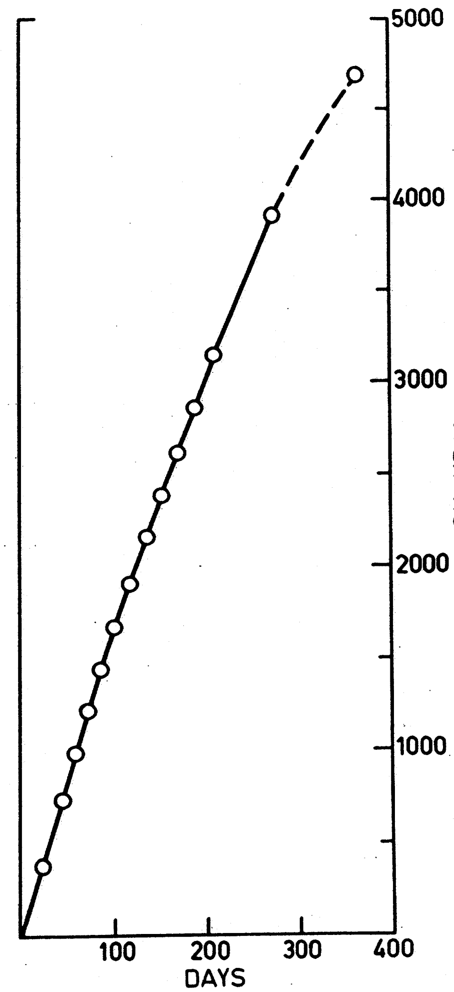

Fig. 1. Milk production of the heifer-cow Lila on a feed described in the text.

Our thanks are due to Masonite Corporation, Laurel, Mississippi for the dried Masonex preparation used in the feeding experiment and to G.A. Serlachius Oy, Lielahti, for the 0-fibre also used in the experiment.

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